ABSCISIN II AND SOME HORMONE-REGULATED PLANT RESPONSES

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Summary

The activity of (±)-abscisin II [(±)AbII] and its interaction with a number of plant growth regulators in the control of lettuce germination, lettuce hypocotyl and radicle elongation, cucumber seedling growth, radish leaf senescence, and barley vernalization were examined. The interactions between (±)AbII and the promoting hormones were of two general types. In gibberellic acid (GA_3)-promoted lettuce germination and kinetin-controlled leaf senescence, the effects of low concentrations of (±)AbII were completely overcome by high concentrations of the other substance. In the second type of interaction, (±)AbII was inhibitory only in the presence of high concentrations of the promoter (kinetin in lettuce germination and allogibberic acid in lettuce radicle elongation). Cucumber radicle elongation, on the other hand, was promoted by (±)AbII in the presence of a mixture of GA_4 and GA_7.

These various responses suggest that linking the mechanism of AbII action with that of one growth hormone alone may be too restrictive.

I. Introduction

The involvement of naturally occurring inhibitory substances in the regulation of plant growth and development has been often suggested, particularly in relation to dormancy, and several substances have been implicated (Evanari 1949; see also Bentley 1958). However, it was only with the isolation of abscisin II (AbII) from immature cotton (*Gossypium hirsutum*) fruits (Ohkuma *et al.* 1963) that an inhibitory substance active at concentrations comparable with the known plant hormones was obtained.

It has since been shown that AbII is the active inhibitor in sycamore (*Acer pseudoplatanus*) leaves (Cornforth *et al.* 1965) and that it is present in a number of other plants (Cornforth, Milborrow, and Ryback 1966). It has been suggested that AbII has a hormonal function in cotton fruit abscission (Addicott *et al.* 1964) and regulates the photoperiodic control of dormancy in birch (Eagles and Wareing 1963, 1964). In addition, AbII inhibits flowering in some long-day plants (Evans 1966; El-Antably, Wareing, and Hillman 1967) and promotes it in some short-day plants, accelerates the senescence of leaf disks from many species, and inhibits bud

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growth on potato tubers though promoting tuber formation on potato plants (El-Antably, Wareing, and Hillman 1967).

The possible importance of the abscisins in the regulation of plant growth has led to experiments, and some speculation, on its mode of action. Addicott *et al.* (1964) demonstrated inhibition of auxin-mediated *Avena* coleoptile growth by AbII, and Thomas, Wareing, and Robinson (1965) found that such inhibition could be overcome by gibberellic acid (GA_3) but not auxin, although the coleoptile is responsive to auxin in the absence of AbII. Thomas, Wareing, and Robinson (1965) also found that their preparations of AbII reduced the elongation of tall (but not dwarf) maize (*Zea mays*) leaf sections and that this inhibition could be overcome by GA_3. In peas (*Pisum sativum*), however, it was the response to GA_3, not normal growth, which was inhibited by these extracts. In another GA_3-controlled response, the release of α-amylase by the barley aleurone layer, AbII was also inhibitory although the inhibition was not clearly competitive (Chrispeels and Varner 1966). Some of these responses could conceivably be due to inhibition of GA_3 synthesis, as has been suggested for the growth retardants (Paleg *et al.* 1965). A similar interpretation was suggested by the inverse relationship between AbII activity and gibberellin activity in sycamore and birch seedlings, although Thomas *et al.* (1965) found no effect of their extracts on GA_3 production by *Gibberella fujikuroi*.

The spectrum of AbII activities led Thomas *et al.* (1965) to suggest that it acts as a gibberellin antagonist *in vivo*, although they reported inhibition with sycamore cambial cell culture which was not reversed by GA_3 and only partially reversed by indoleacetic acid or kinetin. The question of whether AbII is specifically inhibitory to GA_3-controlled responses or whether it inhibits responses controlled by other gibberellins or by other classes of hormones is of importance in evaluating its function in the plant. In the present investigation the activity of AbII has been assessed in responses promoted by GA_3, by other gibberellins and related compounds, by GA_3 or kinetin, or by kinetin alone.

II. Methods

Synthetic ($\pm$)-abscisin II [($\pm$)AbII] was used in all experiments. The sample was obtained through the courtesy of J. van Overbeek and Shell Research Ltd., Sittingbourne, Kent; it is considered a mixture of two enantiomorphs (Cornforth, Milborrow, and Ryback 1965). Allogibberic acid, GA_3, and a mixture of GA_4 and GA_7 were obtained through the courtesy of Imperial Chemical Industries Ltd.

(*a*) *Lettuce Germination*

In all the lettuce germination experiments, 50 seeds (*Lactuca sativa* cv. Great Lakes) were placed on one filter paper in a 5-cm Petri dish with 1 ml of test solution per dish, at least two dishes being used per treatment. Germination, in the dark or light, was at 25°C and was recorded after 48 hr unless otherwise stated. Far-red light was provided by filtering incandescent light through a Westlake FRF 700 plastic filter (250 W incandescent source at 2 ft from seeds); red light was provided by a Philips 40 W red fluorescent tube (2 ft from seeds). A seed was recorded as having germinated when the tip of the radicle was visible. In one experiment in which

seeds were exposed to (±)AbII for limited periods, (±)AbII was removed from these seeds at the end of the exposure period by rinsing three times in distilled water.

(*b*) *Lettuce Hypocotyl and Radicle Extension*

Lettuce seeds (cv. Great Lakes) were pregerminated for 48 hr in the dark at 20°C. At the end of this period, seedlings were selected for uniformity and placed, 10 per 10-cm Petri dish, on two filter papers and 4 ml of test solution. The dishes were placed in clear plastic sleeves to prevent evaporation and incubated for a further 72 hr at 25°C in light (Philips 40 W fluorescent tubes, 900 f.c.). At the end of this period, hypocotyls and radicles were measured to the nearest millimetre. Replication of dishes was threefold and mean values were used in all statistical calculations.

(*c*) *Cucumber Hypocotyl and Radicle Extension*

Cucumber seed (*Cucumis sativus* cv. Long Green) were pregerminated in distilled water at 30°C in light (as for lettuce experiments) for 72 hr. The seedlings were then selected for uniformity and transferred to filter paper in plastic dishes, 20 per dish, with 20 ml of test solution. The dishes were covered with a sheet of glass and incubated in light for a further 48 hr, when the hypocotyls and radicles of the seedlings were measured to the nearest millimetre. Replication was fourfold.

(*d*) *Senescence of Leaf Disks*

Mature, but not senescent, leaves of radish (*Raphanus sativus* cv. Icicle) plants grown in a glasshouse were selected for uniformity. Disks (1 cm diam.) were cut from these leaves, avoiding main veins, and five disks were placed on filter paper in 5-cm Petri dishes with 1 ml of test solution. The disks were incubated in the dark at 20°C for 48 or 96 hr. Chlorophyll was extracted by briefly boiling the disks with two 4-ml portions of 80% ethanol. The extracts were adjusted to 5 ml with 80% ethanol and their absorbances determined at 660 mμ. Each treatment was replicated at least twice.

(*e*) *Vernalization of Barley*

Barley grains (*Hordeum vulgare* cv. Pioneer), which requires 6 weeks vernalization for maximum promotion of flowering (Aspinall, unpublished data), were germinated at 20°C for 12 hr in the presence of GA_3 and (±)AbII [all combinations of 0, 1, 10, and 100 μg/ml GA_3 with 0, 0·25, 2·5, and 25 μg/ml (±)AbII]. At the end of this period the grains were surface-dried, weighed, and placed in tared plastic Petri dishes. Aliquots of the GA_3 or (±)AbII or both solutions were then added to the appropriate dishes to bring the grains to 55% moisture content. The dishes were placed at 3°C for 5 weeks, distilled water being added weekly to maintain the original water content. Following vernalization, the grains were transplanted into pots of soil in a glasshouse. During plant growth the temperature of the glasshouse was maintained above 15°C, and the plants were illuminated continuously with incandescent light (*c.* 100 f.c.). Plants were harvested after 3 and 7 weeks growth and the apices were dissected to observe the extent of floral development.

III. Results

(*a*) *Lettuce Germination*

Although the nature of the response is still uncertain, lettuce seed germination has been shown to be influenced by a variety of factors including red light, gibberellins, and cytokinins. Thus the seed provides a valuable system for evaluating abscisin as an inhibitor of the action of several promotive agents. The cultivar used, Great Lakes, normally germinates well in darkness and is unresponsive to red light unless the seeds are previously inhibited by exposure to far-red light.

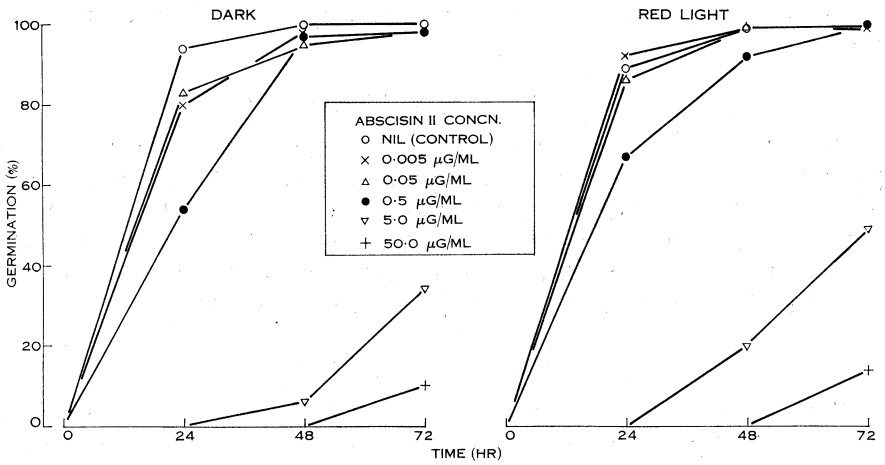

Fig. 1.—(±)-Abscisin II and the germination of lettuce (cv. Great Lakes) seed in the dark or in continuous red light.

Seeds were placed in red light or darkness at various concentrations of (±)AbII and germination was recorded daily under green light for 3 days. Under both conditions the lower concentrations slightly retarded germination (Fig. 1). At the higher concentrations germination was strongly inhibited but there was some germination (12%) by 72 hr, suggesting that the inhibition was not permanent. There was no evidence of an interaction between the effects of (±)AbII and red light.

An attempt was made to associate the inhibitory action of (±)AbII with definite periods of the germination process. Imbibed seeds were placed under far-red light for 48 hr to inhibit germination and some treatments were then illuminated for 5 min with red light. All treatments were then placed in the dark for a further 24 hr. During the experiment the seeds were exposed either for long periods (48–72 hr) to 0·5 μg/ml (±)AbII or for short periods (2 hr) to a higher concentration (5 μg/ml). Table 1 gives a description of the treatments and the resulting germination. Far-red light drastically reduced germination. (±)AbII at both concentrations and at all times significantly inhibited germination unless applied after 50 hr. This indicates that the abscisin-sensitive process(es) are probably completed by 2–4 hr after red light exposure. The inhibition caused by (±)AbII applied prior to the red light may

indicate that the substance was not completely removed by the washing procedure or that the inhibited seed only partially recovered the ability to germinate.

The interaction of the effects of AbII with GA_3 or kinetin was explored by exposing seed to far-red light for 24 hr in the presence of (±)AbII and GA_3 or kinetin and then placing them in the dark for a further 24 hr. High (±)AbII levels completely

TABLE 1

(±)-ABSCISIN II AND SOME HORMONE-REGULATED PLANT RESPONSES

Germination of lettuce (cv. Great Lakes) seeds following different periods of exposure to (±)-abscisin II. All seeds were held for 48 hr in far-red light followed in some cases by 5 min illumination by red light and then a further 24 hr in darkness

Red Light Treatment	Period of Exposure to (±)-Abscisin II (hr from start of expt.)	(±)-Abscisin II Concentration (μg/ml)	Germination at 72 hr (%)	
−	0	0	8·6	
−	0–72	0·5	5·4	
−	0–48	0·5	9·2	
+	0	0	76·1	
+	0–72	0·5	60·2	‡
+	0–48	0·5	61·7	‡
+	0–48*	0·5	63·5	‡
+	46–48	5	64·3	‡
+	47–49	5	65·3	‡
+	48–50†	5	62·0	‡
+	50–52	5	70·9	§
+	52–54	5	77·9	§

* Including red light illumination.

† Excluding red light illumination.

‡ These values significantly different ($P < 0·05$) from value obtained in red light but in absence of (±)AbII.

§ These values not significantly different from value obtained in red light but in absence of (±)AbII.

suppressed the response to all GA_3 concentrations (Fig. 2). Lower levels were less effective. Kinetin was more efficient than GA_3 in reversing the far-red inhibition of germination (Fig. 3). The two lower (±)AbII concentrations showed no significant counteraction of the kinetin promotion although an intermediate concentration was slightly effective, and the highest concentration suppressed the response to kinetin.

(*b*) *Lettuce Hypocotyl and Radicle Extension*

The inhibition of lettuce hypocotyl elongation by light can be overcome by GA_3. On the other hand, radicle elongation is relatively insensitive to GA_3 but is promoted by an allied compound, allogibberic acid (Paleg *et al.* 1964). The interaction of (±)AbII with GA_3 and allogibberic acid in the control of these responses provided information on the comparative effectiveness of (±)AbII and led to a valuable comparison with its effects on germination.

Hypocotyl elongation produced by high levels of GA_3 (Fig. 4) was inhibited only 20% by the highest level of (±)AbII. There was no response to (±)AbII in the absence of GA_3.

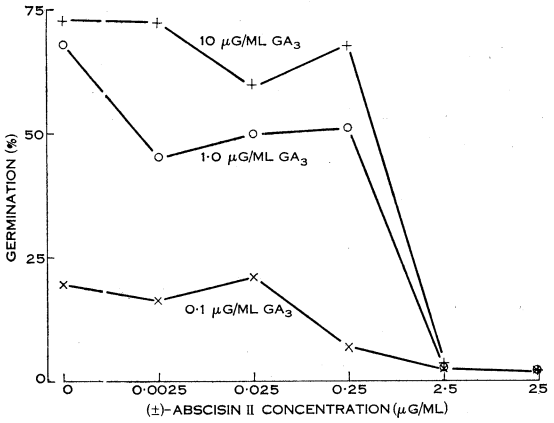

Fig. 2.—Interaction between gibberellic acid (GA_3) at the concentrations indicated and (±)-abscisin II in the control of far-red inhibited lettuce seed germination.

The intermediate, but not the high, GA_3 concentration stimulated radicle elongation when compared with water controls [19·7 and 16·8 mm for GA_3 concentrations of 1 μg/ml and 100 μg/ml respectively; water control, 15·4 mm

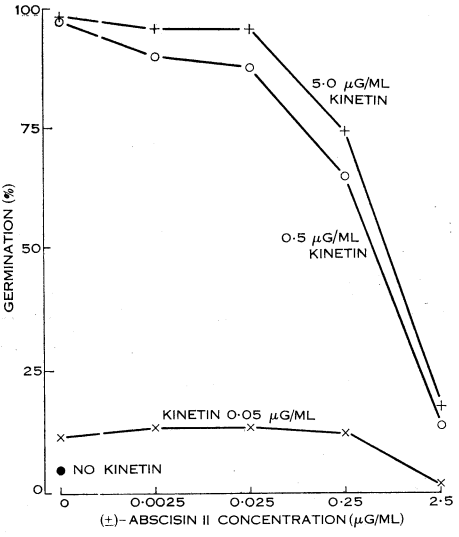

Fig. 3.—Interaction between kinetin and (±)-abscisin II in the control of far-red inhibited lettuce seed germination.

significant difference 1·5 mm ($P = 0·05$)—see Fig. 5]. With both the water controls and the GA_3-treated lettuce seedlings, (±)AbII stimulated radicle elongation at the lower and retarded it at the highest concentration, and there was no evidence of a statistically significant interaction between (±)AbII and GA_3. Radicle elongation

was inhibited rather more by the (±)AbII than hypocotyl elongation in the same seedlings, but this may be merely a manifestation of the greater growth potential of the radicle.

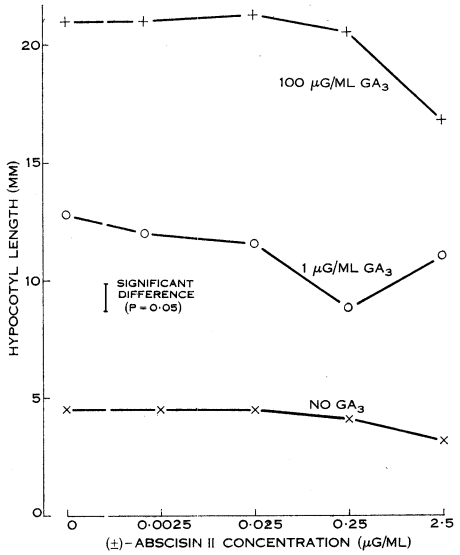

Fig. 4.—Interaction between gibberellic acid (GA_3) at the concentrations indicated and (±)-abscisin II in the control of hypocotyl elongation of lettuce grown in light.

In an initial experiment with allogibberic acid and (±)AbII, lettuce seeds were germinated in the dark for 48 hr before being transferred to the test solutions and incubated in the light for a further 3 days. With both radicles and hypocotyls,

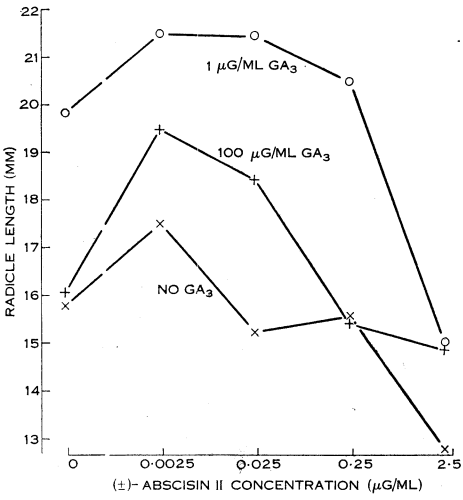

Fig. 5.—Interaction between gibberellic acid (GA_3) at the concentrations indicated and (±)-abscisin II in the control of radicle elongation of lettuce grown in light.

only the (±)AbII at a concentration of 2·5 μg/ml inhibited elongation. In a further experiment, the seeds were grown in the light throughout in an attempt to maximize the effects of allogibberic acid. Allogibberic acid at higher concentrations stimulated

radicle elongation (Fig. 6), but the higher concentrations of (±)AbII were inhibitory. In this case, low concentrations of both allogibberic and (±)AbII produced a slight synergistic promotion of elongation.

(c) *Cucumber Hypocotyl and Radicle Elongation*

The interactions between (±)AbII and GA_3 and allogibberic acid in the lettuce seedling suggested that exploration of a similar system sensitive to another gibberellin also might be worth while. Accordingly the effects of (±)AbII on the growth of

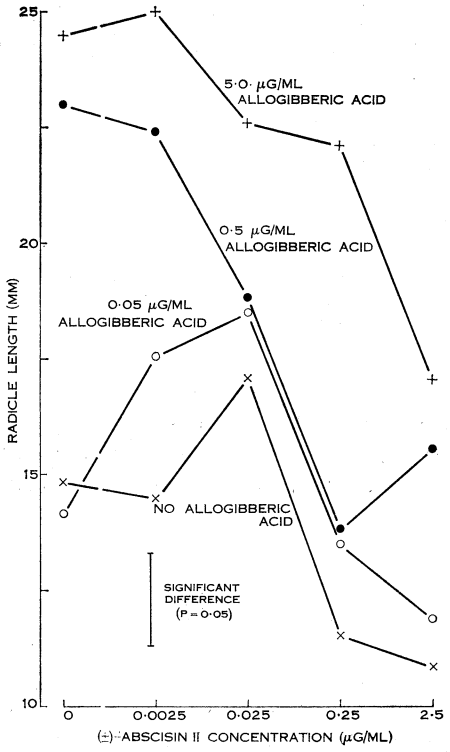

Fig. 6.—Interaction between allogibberic acid and (±)-abscisin II in the control of radicle elongation of lettuce grown in light.

cucumber seedlings in the presence of a mixture of GA_4 and GA_7 was examined. This mixture produced significant hypocotyl elongation (Fig. 7) and, in this system also, the gibberellins interacted with (±)AbII. In the absence of, or at low concentrations of, the gibberellins, the abscisin slightly inhibited elongation. However, at higher gibberellin concentrations, it markedly promoted elongation. This response is in contrast with the effect of GA_3 and (±)AbII on lettuce hypocotyls (Fig. 4).

Cucumber radicle elongation (Fig. 8) was even more markedly stimulated by low levels of (±)AbII than was cucumber hypocotyl elongation. The interaction between the abscisin and the gibberellins did not attain statistical significance although, as with the hypocotyls, the promotive effects of the abscisin were most marked in the presence of gibberellins; indeed, in its absence, elongation was

inhibited by the gibberellins. In this tissue, then, we have the interesting situation of abscisin overcoming the growth *inhibiting* effects of gibberellins.

(*d*) *Senescence of Leaf Disks*

The experiments with lettuce germination showed a kinetin-promoted response inhibited by abscisin. Therefore, it was of interest to confirm this result in a very different system, sensitive to cytokinin, but in which growth and cell division are

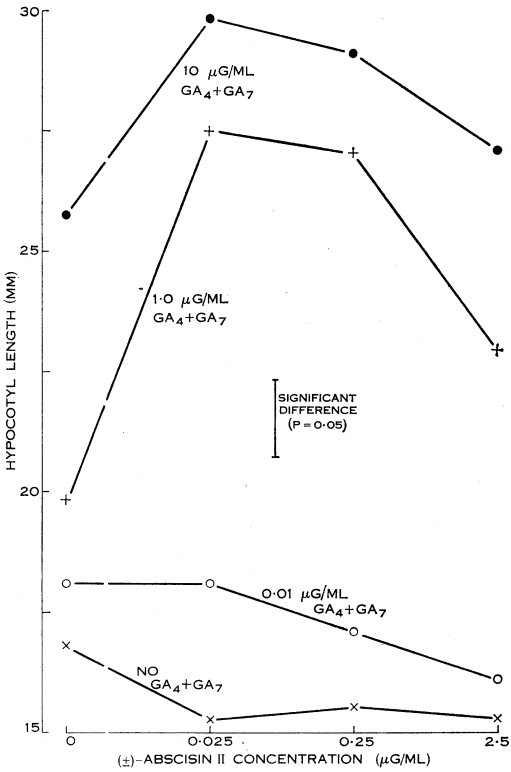

Fig. 7.—Control of cucumber hypocotyl extension by GA_4+GA_7 mixture and (±)-abscisin II.

apparently not involved. Accordingly the interaction of the effects of kinetin and of (±)AbII on chlorophyll retention in radish leaf disks was examined (Loeffler and van Overbeek 1964).

In the initial experiment leaf disks were incubated for 96 hr in the dark at 20°C. There was considerable senescence in the disks incubated in water alone (Fig. 9) and this was not greatly increased by (±)AbII, possibly because chlorophyll loss was virtually complete even in the control series. Kinetin led to the retention of chlorophyll; this response was counteracted by (±)AbII at low kinetin concentrations but not at the higher kinetin concentrations. Thus it appears that the acceleration of senescence by (±)AbII can be overcome by kinetin. In a further experiment over a shorter time interval (48 hr), kinetin alone had only a small effect

on chlorophyll retention (Fig. 10) as little senescence occurred in the control disks. However, (±)AbII reduced chlorophyll retention at low kinetin levels, confirming the results of the first experiment.

In view of the evidence that abscisin can produce GA_3 sensitivity in tissue usually unresponsive to gibberellin (Thomas *et al.* 1965), the response of leaf disks incubated for 48 hr in (±)AbII and GA_3 was explored. (Fig. 11). GA_3 clearly over-

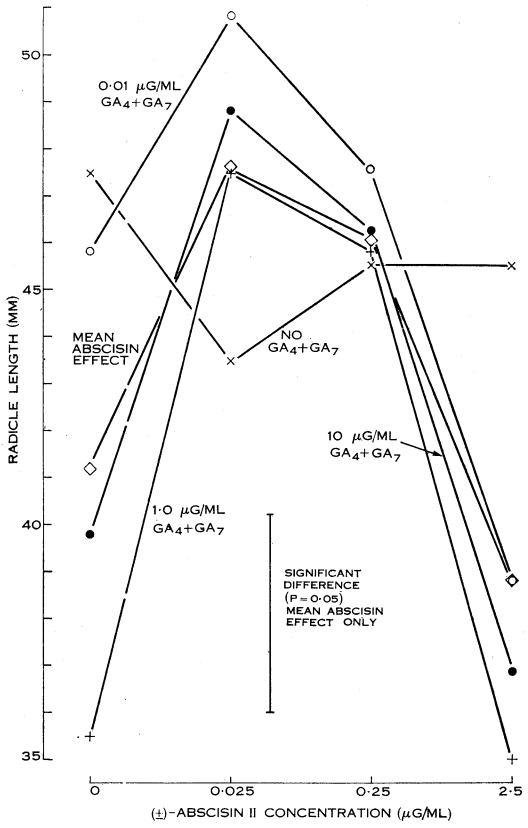

Fig. 8.—Control of cucumber radicle elongation by GA_4+GA_7 mixture and (±)-abscisin II.

came the loss of chlorophyll due to the abscisin. In this and in a further experiment with an extended range of GA_3 concentrations incubated for 72 hr, there was no indication of retardation of leaf disk senescence by GA_3 in the absence of (±)AbII.

(*e*) *Vernalization of Barley*

The growth of barley seedlings shortly after emergence was considerably affected by pretreatment with GA_3 (e.g. first leaf length: 0 GA_3, 11·7 cm; 1 μg GA_3, 15·0 cm; 10 μg GA_3, 15·2 cm; 100 μg GA_3, 17·0 cm) but there was no measurable effect of (±)AbII. This response pattern continued throughout the growth of the plants. At the final harvest (7 weeks after sowing), (±)AbII had had no consistent effect on any of the parameters measured: stage of development, number of leaves on main axis, apex length, and stem length. Although GA_3 had very little

effect on stage of inflorescence development or the number of leaves on the main axis, apex length was reduced (0 GA_3, 35 mm; 100μ g/ml GA_3, 30 mm) as was stem length

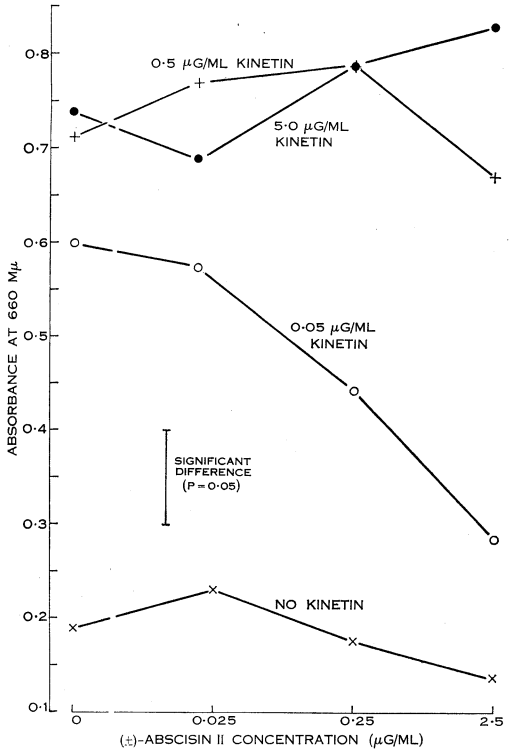

Fig. 9.—Interaction between kinetin and (±)-abscisin II in the retardation of chlorophyll degradation in excised radish leaf disks. Disks incubated for 96 hr in darkness.

(0 GA_3, 34·2 cm; 100 μg/ml GA_3, 29·7 cm). These small, but statistically significant, effects of high GA_3 concentration resemble the inhibitory effects of GA_3 on flowering

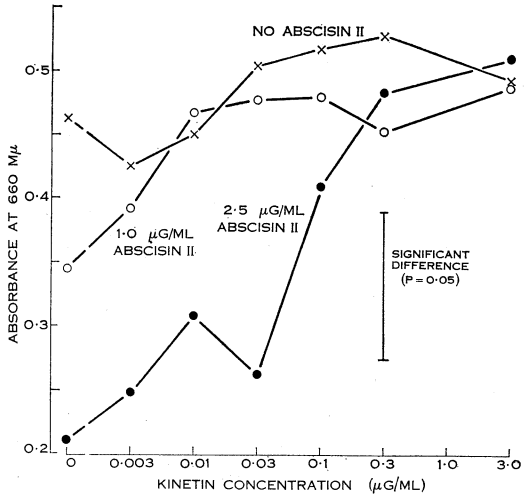

Fig. 10.—Interaction between kinetin and (±)-abscisin II in the retardation of chlorophyll degradation in excised radish leaf disks. Disks incubated for 48 hr in darkness.

in barley previously described (Paleg and Aspinall 1958). There was no evidence that these treatments with (±)AbII or GA_3 had any effect on the vernalization process.

IV. Discussion

The present results add considerably to the spectrum of responses to (±)AbII, particularly the inhibition responses. In our experiments (±)AbII inhibited processes promoted by GA_3 (lettuce hypocotyl growth), other gibberellins (cucumber hypocotyl growth), gibbanes (lettuce radicle growth), gibberellin or cytokinin (lettuce germination), or cytokinin alone (radish leaf senescence). This wide range of inhibitory activity may indicate that (±)AbII does not specifically inhibit one particular hormone-controlled process. The earlier suggestion that the substance acts as a gibberellin antagonist *in vivo* (Thomas *et al.* 1965), although attractive, would seem to be inadequate in view of this range of activity. Although it is possible that a

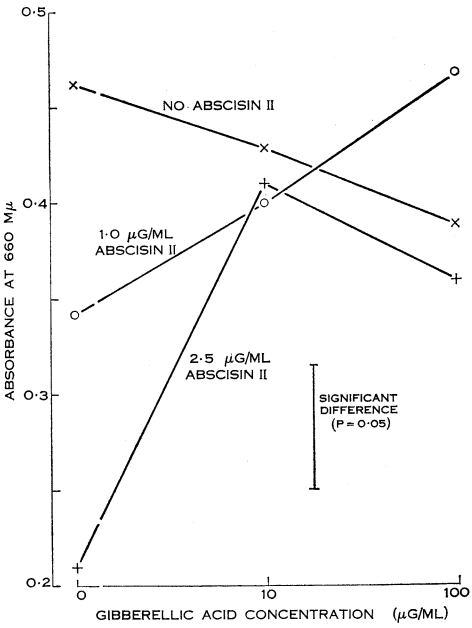

Fig. 11.—Interaction between gibberellic acid and (±)-abscisin II in the retardation of chlorophyll degradation in excised radish leaf disks. Disks incubated for 48 hr in darkness.

gibberellin-controlled step is involved in the responses mediated by the other hormones, such an explanation would require that the effects of abscisin be reversed by gibberellin only. This is not so in radish leaf senescence where both kinetin and GA_3 are effective.

The interactions between (±)AbII and the promoting hormones were of two types in the processes we investigated. In (1) GA_3-controlled lettuce germination and (2) kinetin-controlled leaf senescence, the effects of low concentrations of (±)AbII were completely overcome by high concentrations of the promoting substance. In the second type of interaction, (±)AbII was inhibitory only in the presence of high concentrations of the promoter in (1) lettuce germination controlled by kinetin, and (2) lettuce-radicle elongation controlled by allogibberic acid. Similar interactions have been reported by Thomas *et al.* (1965); the first type in the elongation of tall

maize leaf sections, and the release of reducing sugars from the barley endosperm; the second in pea seedling elongation. It is apparent that neither the process nor the growth promoter involved is specific to the type of abscisin interaction.

Abscisin II is not unique as a naturally occurring growth-inhibiting substance; indeed many such substances have been reported (Evanari 1949). Of these inhibitors coumarin and naringenin are perhaps best known, and it is of interest to compare their activities with those of (±)AbII. Coumarin, which has been isolated from a number of plants (Evanari 1949), will inhibit germination in lettuce seeds, and this inhibition can be overcome by light (Nutile 1945), GA_3 (Mayer 1959), a combination of red light and kinetin (Khan and Tolbert 1965), kinetin alone (Khan and Tolbert 1965), or cycocel (Khan and Tolbert 1966), depending upon the variety of lettuce and the conditions of the experiment. On the other hand, coumarin inhibition of lettuce hypocotyl extension does not seem to be reversed by GA_3 (Mayer 1959; Phillips 1962). Coumarin will also inhibit the release of reducing sugars from cereal endosperm under the influence of GA_3, this inhibition not being reversed by higher GA_3 concentrations (Paleg 1963). Coumarin is not as potent an inhibitor of lettuce germination as (±)AbII; 10^{-4}M coumarin reduced germination in the dark to 13·5% (Mayer 1959) whereas 2×10^{-6}M (±)AbII reduced germination to 6% in the present experiments.

Naringenin, another flavonoid inhibitor, has been found in dormant peach flower buds (Hendershott and Walker 1959). The interactions between naringenin and auxin and gibberellin have been investigated by Phillips (1962) using several growth assays.

In discussing the role of inhibitors in plant processes, stress is frequently laid on a specific competitive action of the inhibitor under discussion. In this connexion (±)AbII, coumarin, and naringenin each seem to approach a competitive relationship with GA_3 in the control of lettuce germination, at least insofar as the effects of a low abscisin concentration can be overcome by higher GA_3 concentrations. On the other hand, lettuce hypocotyl extension was inhibited in a non-competitive manner by each compound. It would appear that competitive or non-competitive inhibition is as much a characteristic of the biological system under consideration as of the precise inhibitor-hormone combination. This is particularly evident since the effects of (±)AbII in leaf disk senescence can be overcome by a completely different substance: kinetin.

Though there are obvious similarities in the actions of the three inhibitors there are also evident differences. (±)AbII seems to be effective at considerably lower concentrations than the others, and its structure suggests that its biosynthetic pathway involves the linking together of isoprenoid units. These features emphasize the possibility that, in fact, AbII may be the forerunner of a new group of biologically important controlling substances.

Further, in complex systems, such as hormone-promoted growth responses, where the parameter measured may be a multiplicity of steps removed from the mechanism of hormone action, the steps at which an inhibitor may produce an effect are numerous, and there is no *a priori* reason for believing that an inhibitor will

always produce its effect at the same step in all responses elicited by that hormone. Thus, in considering the role of an endogenous growth inhibitor such as abscisin, it may even be too restrictive to link its action solely with that of one growth promoter.

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